Protein Changes Related to Ham Processing Temperatures I. Effect of Time-Temperature on Amount and Composition of Soluble Proteins

SUMMARY

Knowledge of protein changes produced by heat is basic to the development of a needed analytical method for determining whether canned meats have been pasteurized at high enough temperatures. Ground cured uncooked ham was heated, with different programming, to temperatures up to 165°F; and the influence of the treatments on the amount and composition of proteins soluble in 0.9% NaCl solution was determined. Rate of heating as well as the temperature reduced the amount of extractable protein. Fractionation of soluble proteins by disc electrophoresis showed five components to be relatively resistant to heat coagulation. One of these components was identified as an acid phosphatase.

INTRODUCTION

In quality control and regulatory work there exists a need for an easily applied simple laboratory technique for determining the temperature to which a heat-treated meat product has been processed. This is very important with products imported into the United States, to ensure that the temperatures applied are high enough to meet requirements. With domestic products also, there are regulations which require heating to, or above, specified temperatures. Quality-control laboratories in industry should often make similar determinations, both to assure compliance with regulations and to maintain quality standards.

A method of determining maximum temperature attained in heat processing has been devised in this country, although not published, and applied both here and in some European laboratories. It depends on heating a solution prepared by extracting the meat product with 0.9% NaCl and observing the temperature at which a flocculent precipitate is formed. Within limits, this temperature corresponds to the highest temperature reached internally when the product is processed. Improvement of the above method, or the development of a new method, to obtain closer correlation between analysis and actual processing temperatures is highly desirable.

The components of meat muscle have been described by Smith (1934, 1937), Szent-Györgyi (1951), Dubuisson (1954), and Mommaerts (1950, 1958). Rabbit myosin is reported to denature in solution when heated to 105–115°F (Smith, 1937), and actomyosin when heated to 113–127°F for 3–12 min (Locker, 1956). Globulin X has been reported to denature on standing at room temperature (Smith, 1937). Myogen, which contains many of the enzymes of glycolysis and of the tricarboxylic acid cycle, denatures, in part, upon standing. However, some of these enzymes are known to be relatively heat-stable. Myoalbumin, which has not been studied to any great extent in the last 30 years, exhibited a series of denaturation "points" at 120, 132, 145, 161, 176, and 194°F (Smith, 1937; Jacob, 1947). In these investigations the solution was filtered free of coagulum (denatured protein) after each temperature was reached, and the heating was continued to higher temperatures until a further coagulum was reached. The multiplicity of denaturation temperatures could possibly be explained by the fact that the myoalbumin used was contaminated with proteins, possibly those generally referred to as myogen.

The above background indicates that myosin, actomyosin, globulin X, and part of the myogen group are denatured at lower temperatures than those used in heat-processing meat. A more accurate knowledge of the heat denaturation of the relatively heat-stable proteins would be useful in developing an index of thermal processing. In preliminary experiments, results showed that temperature and content of proteins soluble in low ionic strength (0.15) were closely related. If not these proteins collectively, identification of one which denatures on heating in the tem-

perature range of processing should be a possibility. For example, phosphatase activity indicates the processing time-temperatures applied to milk (Sanders and Sager, 1947). Data available on the acid phosphatase of meat suggest that it is similarly denatured as a function of time and temperature (Körmendy and Gantner, 1960). Accordingly, an investigation of the heat denaturation of proteins soluble in 0.9% NaCl has been conducted, of which the presently reported study was the initial phase. The present paper deals with changes in the solubility of proteins on heating cured uncooked ham to different temperatures with various rates of heating. The electrophoretic analysis of uncoagulated (relatively heat-stable) components was conducted. One of these components was identified as a muscle acid phosphatase.

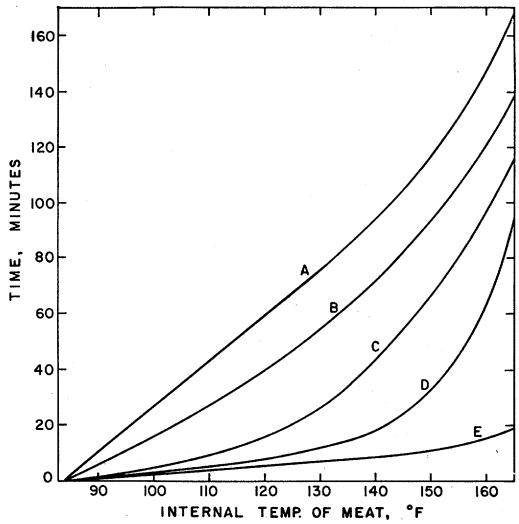

Fig. 1. Time required to obtain selected internal temperatures in cured meat samples by varying initial bath temperatures. Heating for programs A, B, C, D, and E, respectively begun at 84, 100, 120, 140, and 170°F.

EXPERIMENTAL PROCEDURE

Sample preparation. A 15-lb boneless ham was cured at a local processing plant for five days. The arterial pumping method was used to inject 10% by weight of 68° pickle; this was also the strength of the covering pickle. The meat was trimmed free of all but the interstitial fat, ground in a meat grinder in a cold room at 37°F, and then thoroughly mixed. Ground meat was used to minimize the differences in composition and variation of heat penetration that could be expected if samples for analysis were taken from either the whole ham or the individual muscles.

Twenty-five-gram samples, in duplicate, were placed in individual stainless-steel centrifuge cups (approximately 1 × 6 inches), packed tightly, and covered with aluminum foil to minimize evaporation. These samples were heated to an internal temperature of 165°F and samples were withdrawn for analysis at 120, 130, 140, 150, 160, and 165°F. These temperatures correspond to the range that would produce undercooked, properly cooked, and slightly overcooked products in processing. Five heating programs were obtained by starting the cooking bath at 84, 100, 120, 140, and 170°F (Programs A–E, respectively, in Fig. 1), then increasing the bath temperatures to a maximum temperature of 170°F by setting the temperature-control unit to maximum, and heating until the above-mentioned internal temperatures were obtained. The cooked samples were ambient-air-cooled (room temperature 84°F) for 10 min, removed from the tubes, and placed in a 500-ml-capacity two-speed blender with an equal weight of 0.9% NaCl. Each mixture was homogenized for 20 sec (10 sec high speed, and 10 sec low speed), transferred to a beaker, covered with polyethylene film, and held at room temperature for a 30-min extraction. The pH was recorded at this time with a single-probe electrode calibrated at pH 6.5 and 84°F. The mixtures were then centrifuged at 32,800 × G in a refrigerated centrifuge for 30 min. The supernatant fractions, which contained some fat floating on the surface, were removed with a long cannula, and then further clarified in a refrigerated centrifuge at 32°F at 130,000 × G for 30 min. The clear extracts were used for protein determinations and disc electrophoresis.

Protein determination. One-ml fractions were used for protein determinations by the biuret method (Gornall *et al.*, 1949). The biuret standard was a solution of bovine serum albumin (BSA) calibrated by micro-Kjeldahl analysis (Ogg, 1960). The protein was determined by spectrophotometry at 540 mμ after 30 min of color development. Two different concentrations of the BSA, in duplicate, and a biuret blank were read against a distilled-water blank. The color was stable for several hours. The calibration curve (containing six points) was linear to 0.650 absorbance units on an instrument calibrated to be linear from 0.000 to 3.000 absorbance units. Each fraction of meat extract, containing 3–26 mg protein, was run in duplicate, and the results are reported as the mean, with standard deviation of ±0.03 mg.

Disc electrophoresis. Extracts of several samples which had been heated to various temperatures were fractionated by disc electrophoresis on polyacrylamide gels according to techniques of Ornstein (1964) and Davis (1964). These included extracts from unheated samples and samples heated to 150°F at different programs (A,D,E,); also included were samples heated to different temperatures: 120, 150, and 160°F at the same program (D).

Lower gels 2 inches long were used in place of the standard 1½-inch gels, to obtain better separation. The extracts, each containing 200 μg protein, were mixed with upper gel solution at 1:1 instead of the standard 1:50 dilution. This was done because the protein solutions were already dilute and it was necessary to restrict the volume of extracts added to the top of the electrophoretic tube. The 1:1 mixtures were placed on top of the spacer gels and then photopolymerized for 20 min. Electrophoresis was conducted for 30–50 min, depending on the progress of a tracking dye which preceded the fastest-moving protein. To minimize diffusion, the gels were immediately removed from the electrophoretic bath upon completion of the run, placed in an ice bath, removed from the tube with a probing needle, and stained for 1 hr. For complete destaining, the gels were soaked in a large test tube containing 7.5% acetic acid which was changed three times in 36 hr, and were then destained electrophoretically at 10 ma per tube for 40 min. The upper chamber contained a small amount of staining dye to prevent excessive destaining. This destaining method produced a background almost as lightly colored as that found in the area in front of the tracking dye. The absorbance of stained gels was scanned with a recording microdensitometer.

In addition to staining gels for protein (aniline black 0.5% w/v in 3.5% acetic acid), a specific stain for phosphatase was used on duplicate gels of unheated samples and samples heated to 150°F (program E). A modification of the simultaneous-coupling azo-dye method (Barka, 1961) was used. After the gels were removed from the glass tubes, the gels were immersed twice in a pH-5.0 acetate buffer for 15-min periods. The gels were then placed into tubes containing Fast Garnet GBC salt, 1 mg/ml, in pH-5.0 acetate buffer for 30 min. This allowed the dye to penetrate to the center of the gel. Finally, the gels were placed into tubes containing 2 mg/ml of α-naphthol phosphate salt, made in pH 5.0 acetate buffer, for 24 hr at 35°F. The reaction was stopped by placing the gels in 7.5% acetic acid. The sites colored dark red were compared with the gels stained with aniline black to determine the position of the phosphatase. These gels were photographed by transmitted light with a Mazda lamp through a series B filter. High-contrast film and standard development was used.

RESULTS AND DISCUSSION

Fig. 1 shows the time in minutes required to attain various internal temperatures in the heated samples. The curves plotted indicate that the procedure used in heating produced five different programs of heating: A(slow)–E(rapid).

Fig. 2 relates to temperature the amounts of protein that remained soluble in 0.9% NaCl when the samples of ham were heated at the five different programs. Heating reduced the content of soluble proteins from 26.75 mg/ml of extract to 5.10–9.24 mg/ml at 120°F and to 2.60–4.48 mg/ml at 150°F. The proteins remaining soluble after heating were a small fraction of those originally soluble. However, more significantly, the rapid rates of heating tended to increase the amount of protein insolubilized. This is indi-

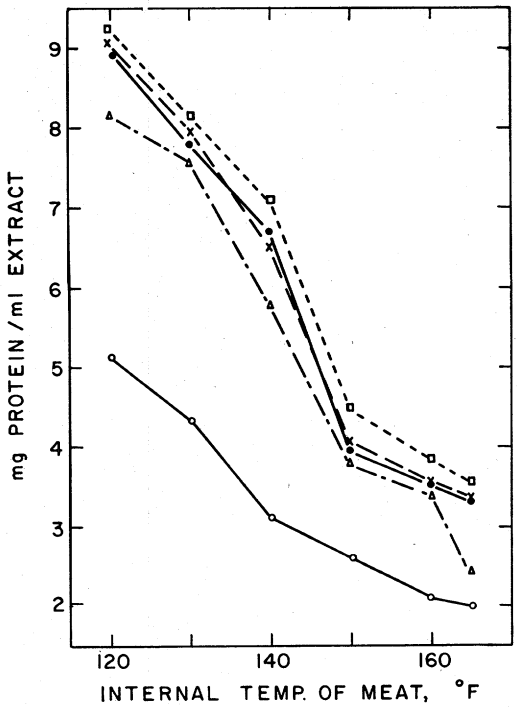

Fig. 2. Soluble protein in meat as affected by heating at different rates and to different temperatures (*cf.* Fig. 1). The protein content of the extracts from the uncooked meat samples at 84°F was 26.75 mg/ml extract. Heating curve: A = □---□; B = x--x; C = ●——●; D = △---△; E = ○——○.

cated by comparison of the protein remaining soluble at corresponding sampling temperatures on curves A–E (Fig. 2). The results show that the amount of protein extractable after heating to a given terminal temperature—for example, 150 or 160°F—varied with the time-temperature combination rather than internal temperature. For example, 3.8 mg soluble protein/ml sample was extractable from samples heated with the five different programs at temperatures of 133, 152.5, 153.5, 154.5, and 161°F. These results indicate that simply determining the protein content of extracts of heat-processed meats can indicate approximate processing temperature, but not with high accuracy.

The pH values of the ham samples investigated were within the normal range of cured heated ham; otherwise, adjustment would have been advisable. The effect of time and temperature on the change of pH of the meat extracts (Table 1) shows that pH increased on heating, as previously reported and explained by Hamm (1960) and Hamm and Deatherage (1960). The observed pH change was not more than 0.3 unit, not as large as reported previously (0.4 pH). This may be accounted for by differences in the buffering capacity of cured and uncured meat, since Hamm's studies were, primarily, on uncured meats. Over 65% of the change occurred on heating to 120°F, and differences produced by increasing temperature above 120°F were 0.07 unit or less. The results indicate that the effects of varying temperature 10–30°F would be expected to produce small pH changes that measurably influence protein solubility only under unusual circumstances.

Fig. 3a shows a disc electrophoretic scan of proteins extractable with a solution having

Table 1. Time and temperature effects on the change of pH of meat samples.[a]

Program of heating[b]	pH of meat at given temperatures (°F)					
	120	130	140	150	160	165
A	5.96	5.96	6.02	6.02	6.02	6.04
B	5.95	5.95	5.95	6.02	6.04	6.05
C	5.95	5.98	6.02	6.02	6.05	6.05
D	5.95	5.95	5.98	6.00	6.02	6.02
E	5.95	5.95	6.02	6.02	6.02	6.02

[a] pH of uncooked meat was 5.75.
[b] *cf.* Fig. 1.

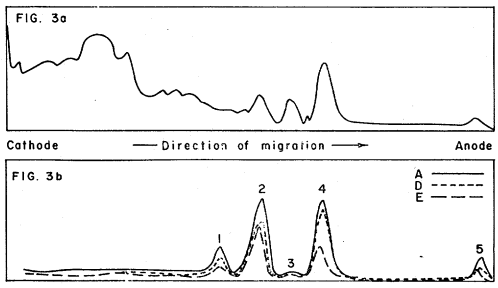

Fig. 3a. Disc electrophoretic pattern of proteins in extract from uncooked meat.
Fig. 3b. Disc electrophoretic patterns of proteins in extracts from meat heated to 150°F at different rates, programs A, D, and E.

an ionic strength of 0.15 from uncooked cured ham, while Fig. 3b shows scans indicating the proteins that remained in samples that were cooked to 150°F with different rates of heating. These electrophoretic patterns show that protein components that were a major fraction of the extract from unheated ham were not present in the heated samples to any appreciable extent. In contrast, there is an increase in the peaks designated as 1–5 after heating to the 150°F range. Each pattern represents fractionation of 200 μg protein. The heat-stable proteins represent an increasingly larger portion of the proteins remaining as heating removed that portion of protein which was heat-coagulable.

The relative size of peaks 1–5 diminished in the order A, D, E, reflecting the tendency of rapid heating rates to heat-coagulate more protein than comparatively slower rates.

Fig. 4 shows the effect of increasing sampling temperature on heating at the same rate (Program D). The scans indicate that peaks 2 and 4 increase to a maximum with heating to 150°F and then decrease at 160°F, thus indicating a heat lability in the 150–160°F range. Peak 5 increases with increased heating, indicating a heat-stable protein, which, because of its fast mobility, may

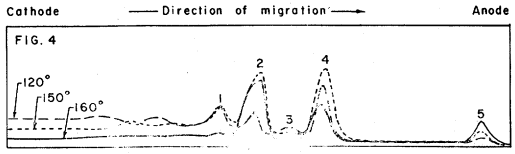

Fig. 4. Disc electrophoretic patterns of proteins in extracts from meat heated to 120, 150, and 160°F at the heating rate of program D.

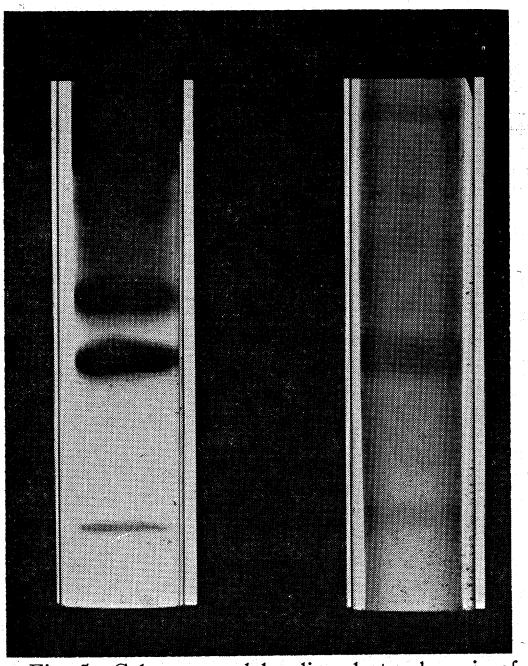

Fig. 5. Gels prepared by disc electrophoresis of meat extract stained (left gel) with aniline black to show location of protein, and (right gel) with Fast Garnet GBC-α-naphthol to show location of acid phosphatase.

be an albumin or a low-molecular-weight fragment derived from one of the proteins initially present. The comparative size of peaks 1 and 3 reduced as temperature increased.

Peaks 1–5 contain proteins apparently of relatively high heat stability.

Fig. 5 is a photograph of disc electrophoretic gels of unheated extracts stained to locate protein or phosphatase. The location of the area reacting positively for phosphatase activity corresponds to peak 4 in Figs. 3a, 3b, and 4. Owing to the relative insensitivity of the dye for locating acid phosphatase (Barka, 1961), the stained areas of the heated samples were barely discernible and not intense enough for photography. Further characterization may indicate the muscle phosphatase identified in the present work to be identical to the preparations investigated by Körmendy.

Further investigation is concerned with the identity and properties of the remaining heat-stable components fractionated by electrophoresis and the correlation of their heat lability with time-temperature conditions in heat processing.

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